D E C L A R A T I O N

I, SHINICHI USUI, a Japanese Patent Attorney registered No. 9694, of Okabe International Patent Office at No. 602, Fuji Bldg., 2-3, Marunouchi 3-chome, Chiyoda-ku, Tokyo, Japan, hereby declare that I have a thorough knowledge of Japanese and English languages, and that the attached pages contain a correct translation into English of the priority documents of Japanese Patent Application No. 2002-309786 filed on October 24, 2002 in the name of CANON KABUSHIKI KAISHA.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 19th day of September, 2008

SHINICHI USUI

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this office.

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Commissioner,
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[Title of the Invention]	NEW POLYHYDROXYALKANOATE COMPRISING UNIT HAVING (PHENYLEMTHYL)OXY STRUCTURE ON SIDE CHAIN, AND METHOD FOR PRODUCING THE SAME
[Number of the Claims]	20

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[Material] Drawings 1

[Material] Abstract 1

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[Title of the Invention]

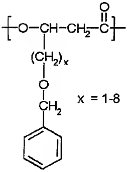
NEW POLYHYDROXYALKANOATE COMPRISING UNIT HAVING (PHENYLMETHYL)OXY STRUCTURE ON SIDE CHAIN, AND METHOD FOR PRODUCING THE SAME

[Claims]

[Claim 1]

A polyhydroxyalkanoate comprising a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit represented by a chemical formula (1):

[chemical 1]



(1)

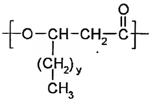
(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula).

[Claim 2]

The polyhydroxyalkanoate according to claim 1, comprising, in addition to the unit represented by the chemical formula (1), at least one of the units

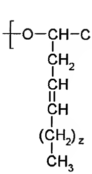
represented by chemical formulae (2) and (3):

[chemical 2]



y = 1-8

(2)



z = 3,5

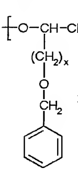
(3)

(wherein y and z each may assume one or more arbitrary integral values within the range shown in the chemical formula, independently from the unit represented by the chemical formula (1)).

[Claim 3]

The polyhydroxyalkanoate according to claim 1 or 2, comprising, simultaneously in a molecule thereof, at least the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit represented by the chemical formula (1):

[chemical 3]



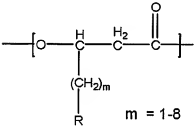
x = 1-8

(1)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula);

and a 3-hydroxy-ω-cyclohexylalkanoic acid unit represented by a chemical formula (4):

[chemical 4]

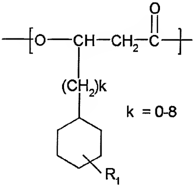


(4)

(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R includes a residue having either a phenyl structure or thienyl structure),

or represented by a chemical formula (5):

[chemical 5]



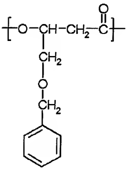
(5)

(wherein R_1 represents a substituent on a cyclohexyl group and R_1 is a H atom, a CN group, a NO_2 group, a

halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and k may assume one or more arbitrary integral values within the range shown in the chemical formula).

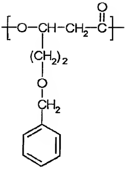
[Claim 4]

The polyhydroxyalkanoate according to any one of claims 1 to 3, wherein the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit represented by the chemical formula (1) is either one or more of:
a 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid unit represented by a chemical formula (6):
[chemical 6]



(6)

and a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit represented by a chemical formula (7):
[chemical 7]

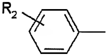


(7)

[Claim 5]

The polyhydroxyalkanoate according to claim 3 or 4, wherein R in the chemical formula (4), namely the residue having a phenyl structure or a thienyl structure belongs to a group of residues represented by a chemical formula (8):

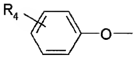
[chemical 8]



(8)

(wherein R_2 indicates a substituent group on the aromatic ring and R_2 represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $CH=CH_2$ group, a $COOR_3$ group (wherein R_3 represents any one of a H atom, a Na atom and a K atom), a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_2 may be different for each unit); a group of residues represented by a chemical formula (9):

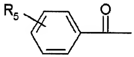
[chemical 9]



(9)

(wherein R_4 indicates a substituent group on the aromatic ring and R_4 represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a SCH_3 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_4 may be different for each unit; a group of residues represented by a chemical formula (10):

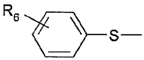
[chemical 10]



(10)

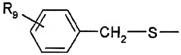
(wherein R_5 indicates a substituent group on the aromatic ring and R_5 is a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_5 may be different for each unit); a group of residues represented by a chemical formula (11):

[chemical 11]



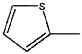
(11)

(wherein R_6 indicates a substituent group on the aromatic ring and R_6 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_7$ group, a SO_2R_8 group (wherein R_7 represents any one of H, Na, K, CH_3 and C_2H_5, and R_8 represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2$-CH group, or a $(CH_3)_3$-C group, and in a case where plural units are present, R_6 may be different for each unit); a group of residues represented by a chemical formula (12):
[chemical 12]



(12)

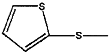
(wherein R_9 represents a substituent group on the aromatic ring, and R_9 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{10}$ group, a SO_2R_{11} group (wherein R_{10} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{11} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2$-CH group or a $(CH_3)_3$-C group, and in a case where plural units are present, R_9 may be different for each unit); a group of residues represented by a chemical formula (13):
[chemical 13]



(13)

a group of residues represented by a chemical formula (14):

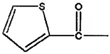
[chemical 14]



(14);

a group of residues represented by a chemical formula (15):

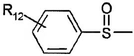
[chemical 15]



(15)

a group of residues represented by a chemical formula (16):

[chemical 16]

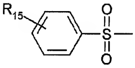


(16)

(wherein R_{12} indicates a substituent group on the aromatic ring and R_{12} represents any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{13}$ group, a SO_2R_{14} group (wherein R_{13} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{14} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3

group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2$-CH group and $(CH_3)_3$-C group, and in a case where plural units are present, R_{12} may be different for each unit); a group of residues represented by a chemical formula (17):

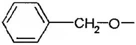
[chemical 17]



(17)

(wherein R_{15} indicates a substituent group on the aromatic ring and R_{15} is any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{16}$ group, a SO_2R_{17} group (wherein R_{16} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{17} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7, group, a $(CH_3)_2$-CH group and a $(CH_3)_3$-C group, and in a case where plural units are present, R_{15} may be different for each unit); and a group of residues represented by a chemical formula (18):

[chemical 18]



(18)

[Claim 6]

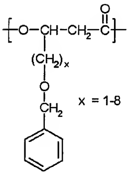
The polyhydroxyalkanoate according to any one

of claims 1 to 5, wherein a number-average molecular weight is within a range from 1,000 to 1,000,000.

[Claim 7]

A method for producing a polyhydroxyalkanoate containing, in a molecule thereof, a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit represented by a chemical formula (1):

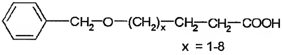
[chemical 21]



(1)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula), which comprises allowing, under a condition containing ω-[(phenylmethyl)oxy]alkanoic acid represented by a chemical formula (19):

[chemical 19]

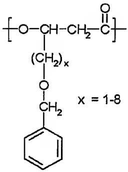


(19)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical

formula), a microorganism having an ability to produce a polyhydroxyalkanoate containing in a molecule thereof a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit of the chemical formula (1):

[chemical 20]



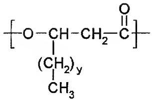
(1)

to execute biosynthesis.

[Claim 8]

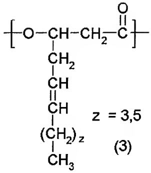
The method for producing a polyhydroxyalkanoate according to claim 7, wherein polyhydroxyalkanoate contains, in addition to the unit represented by the chemical formula (1), at least one of the units represented by chemical formulae (2) and (3):

[chemical 22]



y = 1-8

(2)



z = 3,5

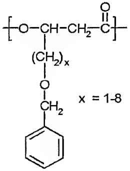
(3)

(wherein y and z each may assume one or more arbitrary integral values within the range shown in the chemical formulae, independently from the unit represented by the chemical formula (1)).

[Claim 9]

The producing method according to claim 7 or 8 for producing a polyhydroxyalkanoate containing, within a molecule at the same time, a 3-hydroxy-ω-[(phenylmethyl)oxy] alkanoic acid unit represented by a chemical formula (1):

[chemical 29]



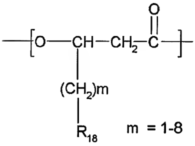
x = 1-8

(1)

(wherein x may assume one or more arbitrary integral

values within the range shown in the chemical formula), and a 3-hydroxy-ω-cyclohexylalkanoic acid unit represented by a chemical formula (22):

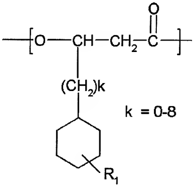
[chemical 30]



(22)

(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R_{18} includes a residue having either a phenyl structure or thienyl structure), or represented by a chemical formula (5):

[chemical 31]

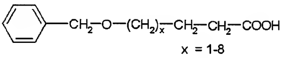


(5)

(wherein R_1 represents a substituent on a cyclohexyl group and R_1 is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and k may

assume one or more arbitrary integral values within the range shown in the chemical formula), the method comprising, under a condition containing ω-[(phenylmethyl)oxy]alkanoic acid represented by a chemical formula (19):

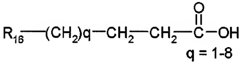
[chemical 23]



(19)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula) and ω-cyclohexylalkanoic acid represented by a chemical formula (20)

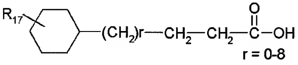
[chemical 24]



(20)

(wherein q may assume one or more arbitrary integral values within the range shown in the chemical formula; and R_{16} includes a residue having either a phenyl structure or thienyl structure), or represented by:

[chemical 25]

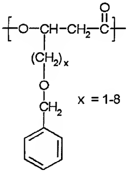


(21)

(wherein R_{17} represents a substituent on a cyclohexyl

group and R_{17} is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and r may assume one or more arbitrary integral values within the range shown in the chemical formula), utilizing ω-[(phenylmethyl)oxy] alkanoic acid represented by the chemical formula (19) and the compound represented by the chemical formula (20) or ω-cyclohexylalkanoic acid represented by the chemical formula (21) as the raw material and executing a biosynthesis by a microorganism having an ability to produce a polyhydroxyalkanoate including, in a molecule thereof at the same time, a 3-hydroxy-ω-[(phenylmethyl)oxy] alkanoic acid unit represented by the chemical formula (1):

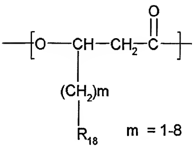
[chemical 26]



(1)

(wherein x may assume one or more arbitrary integral values within the range shown in the chemical formula) and a 3-hydroxy-ω-cyclohexylalkanoic acid unit represented by a chemical formula (22)

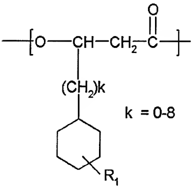
[chemical 27]



(22)

(wherein m may assume one or more arbitrary integral values within the range shown in the chemical formula; and R_{18} includes a residue having either a phenyl structure or thienyl structure), or represented by a chemical formula (5):

[chemical 28]



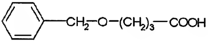
(5)

(wherein R_1 represents a substituent on a cyclohexyl group and R_1 is a H atom, a CN group, a NO_2 group, a halogen atom, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and k may assume one or more arbitrary integral values within the range shown in the chemical formula).

[Claim 10]

The method for producing a polyhydroxyalkanoate according to any one of claims 7 to 9, wherein the ω-[(phenylmethyl)oxy]alkanoic acid represented by said chemical formula (19) is 4-[(phenylmethyl)oxy]butyric acid represented by a chemical formula (23):

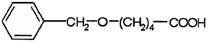
[chemical 32]



(23)

or 5-[(phenylmethyl)oxy]valeric acid represented by a chemical formula (24):

[chemical 33]

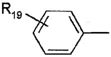


(24).

[Claim 11]

The method for producing a polyhydroxyalkanoate according to claim 9 or 10, wherein R_{16} in the chemical formula (20) and R_{18} in the chemical formula (22), namely the residues having a phenyl structure or a thienyl structure, belong to a group of residues represented by a chemical formula (25):

[chemical 34]

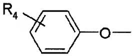


(25)

(wherein R_{19} indicates a substituent group on the

aromatic ring and R_{19} represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $CH{=}CH_2$ group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_{19} may be different for each unit); a group of residues represented by a chemical formula (9):

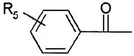
[chemical 35]



(9)

(wherein R_4 indicates a substituent group on the aromatic ring and R_4 represents a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a SCH_3 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group, and in a case where plural units are present, R_4 may be different for each unit); a group of residues represented by a chemical formula (10):

[chemical 36]

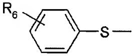


(10)

(wherein R_5 indicates a substituent group on the aromatic ring and R_5 is a H atom, a halogen atom, a CN group, a NO_2 group, a CH_3 group, a C_2H_5 group, a C_3H_7 group, a CF_3 group, a C_2F_5 group or a C_3F_7 group,

and in a case where plural units are present, R_5 may be different for each unit); a group of residues represented by a chemical formula (11):

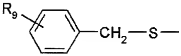
[chemical 37]



(11)

(wherein R_6 indicates a substituent group on the aromatic ring and R_6 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_7$ group, a SO_2R_8 group (wherein R_7 represents any one of H, Na, K, CH_3 and C_2H_5, and R_8 represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2$-CH group, or a $(CH_3)_3$-C group, and in a case where plural units are present, R_6 may be different for each unit); a group of residues represented by a chemical formula (12):

[chemical 38]

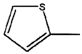


(12)

(wherein R_9 represents a substituent group on the aromatic ring, and R_9 represents a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{10}$ group, a SO_2R_{11} group (wherein R_{10} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{11} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5

group, a C_3H_7 group, a $(CH_3)_2$-CH group or a $(CH_3)_3$-C group, and in a case where plural units are present, R_9 may be different for each unit); a group of residues represented by a chemical formula (13):

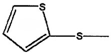
[chemical 39]



(13)

a group of residues represented by a chemical formula (14):

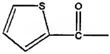
[chemical 40]



(14);

a group of residues represented by a chemical formula (15):

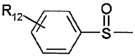
[chemical 41]



(15)

a group of residues represented by a chemical formula (16):

[chemical 42]

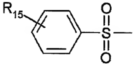


(16)

(wherein R_{12} indicates a substituent group on the

aromatic ring and R_{12} represents any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{13}$ group, a SO_2R_{14} group (wherein R_{13} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{14} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7 group, a $(CH_3)_2$-CH group and $(CH_3)_3$-C group, and in a case where plural units are present, R_{12} may be different for each unit); a group of residues represented by a chemical formula (17):

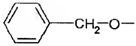
[chemical 43]



(17)

(wherein R_{15} indicates a substituent group on the aromatic ring and R_{15} is any one of a H atom, a halogen atom, a CN group, a NO_2 group, a $COOR_{16}$ group, a SO_2R_{17} group (wherein R_{16} represents any one of H, Na, K, CH_3 and C_2H_5, and R_{17} represents any one of OH, ONa, OK, a halogen atom, OCH_3 and OC_2H_5), a CH_3 group, a C_2H_5 group, a C_3H_7, group, a $(CH_3)_2$-CH group and a $(CH_3)_3$-C group, and in a case where plural units are present, R_{15} may be different for each unit); and a group of residues represented by a chemical formula (18):

[chemical 44]



(18)

[Claim 12]

The method for producing a polyhydroxyalkanoate according to any one of claims 7 to 11, wherein the microorganism is cultured in a medium containing the ω-[(phenylmethyl)oxy]alkanoic acid represented by chemical formula (19).

[Claim 13]

The method for producing a polyhydroxyalkanoate according to any one of claims 9 to 11, wherein the microorganism is cultured in a medium containing the ω-[(phenylmethyl)oxy]alkanoic acid represented by chemical formula (19) and the compound represented by the chemical formula (20) or the ω-cyclohexylalkanoic acid represented by chemical formula (21).

[Claim 14]

The method for producing a polyhydroxyalkanoate according to claim 12 or 13, wherein the microorganism is cultured in a medium containing, in addition to ω-[(phenylmethyl)oxy]alkanoic acid represented by chemical formula (19), at least one of peptides, yeast extracts, organic acids or salts

thereof, amino acids or salts thereof, saccharides and straight-chain alkanoic acids containing 4 to 12 carbon atoms or salts thereof.

[Claim 15]

The method for producing a polyhydroxyalkanoate according to claim 14, wherein the peptide contained in the culture medium is polypeptone; the organic acids contained in the culture medium or salts thereof are one or more compounds selected from the group consisting of pyruvic acid, oxaloacetic acid, citric acid, isocitric acid, ketoglutaric acid, succinic acid, fumaric acid, malic acid, lactic acid, and salts thereof; the amino acids or salts thereof are one or more compounds selected from the group consisting of glutamic acid, aspartic acid, and salts thereof; and the saccharides contained in the culture medium are one or more compounds selected from the group consisting of glyceroaldehyde, erythrose, arabinose, xylose, glucose, galactose, mannose, fructose, glycerol, erythritol, xylitol, gluconic acid, glucronic acid and galacturonic acid, maltose, sucrose and lactose.

[Claim 16]

The method for producing a polyhydroxyalkanoate according to any one of claims 12 to 15, wherein said

culture of microorganisms comprises two or more culturing steps.

[Claim 17]

The method for producing a polyhydroxyalkanoate according to claim 16, wherein said culture is a fed-batch culture.

[Claim 18]

The method for producing a polyhydroxyalkanoate according to any one of claims 12 to 17, comprising a step of culturing the microorganism in a medium containing ω-[(phenylmethyl)oxy]alkanoic acid represented by chemical formula (19) and recovering polyhydroxyalkanoate containing 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit represented by the chemical formula (1) generated by the microorganism from the cells of the microorganism.

[Claim 19]

The method for producing a polyhydroxyalkanoate according to any one of claims 7 to 18, wherein the microorganism belongs to Pseudomonas species.

[Claim 20]

The method for producing a polyhydroxyalkanoate according to claim 19, wherein the microorganism is

one or more strains selected from the group consisting of Pseudomonas cichorii YN2 (FERM BP-7375), Pseudomonas cichorii H45 (FERM BP-7374) and Pseudomonas jessenii P161 (FERM BP-7376).

File No. 4815021

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a polyhydroxyalkanoate comprising a new unit, and a method for producing the same using microorganisms.

[0002]

[Background Art]

It has been reported so far that many types of microorganisms produce poly-3-hydroxybutyric acid (PHB) or other polyhydroxyalkanoates (PHAs) and accumulate them in the cells (see Non-Patent Literature 1). As with the conventional plastics, polymers such as polyhydroxyalkanoate produced by microorganisms are subjected to melting processing, so that they can be used for production of various types of products. Moreover, polymers produced by microorganisms, such as polyhydroxyalkanoates, are biodegradable, and accordingly, they have an advantage in that they are completely decomposed by microorganisms existing in the nature. Accordingly, for example, when a polyhydroxyalkanoate produced by microorganisms is discarded, differing from many conventional synthetic polymer compounds, it does not remain in the environment as is, and therefore it

does not cause pollution. Furthermore, since polyhydroxyalkanoate produced by microorganisms have excellent biocompatibility, it is expected that these compounds will be applied to soft components for medical use, etc.

[0003]

It is known that these polyhydroxyalkanoates produced by microorganisms can have various compositions or structures depending on the type of microorganisms used for production, the composition of a medium, culture conditions, etc. Studies have been made so far to attempt to control the composition or structure of the polyhydroxyalkanoate produced by microorganisms, mainly from the viewpoint of the improvement of physical properties of the polyhydroxyalkanoate.

[0004]

As a study directed towards controlling the composition or structure of the polyhydroxyalkanoate produced by microorganisms, a study has been vigorously made to allow microorganisms to produce polyhydroxyalkanoate having an aromatic ring in its unit in these years.

[0005]

(a) Polyhydroxyalkanoate comprising phenyl group or its partially substituted form

It has been reported that using 5-phenyl

valeric acid as a substrate, Pseudomonas oleovorans produces a polyhydroxyalkanoate comprising 3-hydroxy-5-phenyl valeric acid as a unit (see Non- Patent Literatures 2 and 3).

It has been reported that using 5-(p-tolyl)valeric acid as a substrate, Pseudomonas oleovorans produces a polyhydroxyalkanoate comprising 3-hydroxy-5-(p-tolyl)valeric acid as a unit (see Non-Patent Literature 4).

It has been reported that using 5-(2,4-dinitrophenyl)valeric acid as a substrate, Pseudomonas oleovorans produces a polyhydroxyalkanoate comprising 3-hydroxy-5-(2,4-dinitrophenyl)valeric acid and 3-hydroxy-5-(p-nitrophenyl)valeric acid as units (see Non-Patent Literature 5).

[0006]

(b) Polyhydroxyalkanoate comprising phenoxy group or its partially substituted form

It has been reported that using 11-phenoxy undecanoic acid as a substrate, Pseudomonas oleovorans produces a polyhydroxyalkanoate copolymer comprising a 3-hydroxy-5-phenoxy valeric acid unit and a 3-hydroxy-9-phenoxynonanoic acid unit (see Non-Patent Literature 6).

[0007]

There has been disclosed an invention relating

to a homopolymer consisting of 3-hydroxy-5-(monofluorophenoxy)pentanoate (3H5(MFP)P) units or 3-hydroxy-5-(difluorophenoxyl)pentanoate (3H5(DFP)P) units; a copolymer containing at least (3H5(MFP)P) units or (3H5(DFP)P) units; Pseudomonas putida having an ability to synthesize these polymers; and a method of producing the above described polymers using Pseudomonas species. In addition, it is described as an advantage of the above invention that a long chain aliphatic acid having substituent groups can be assimilated to synthesize a polymer having a phenoxy group substituted with 1 or 2 fluorine atoms at the side chain terminal and that the polymer provides stereoregularity and water repellency, while maintaining a high melting point and good processability (see Patent Literature 1).

[0008]

Moreover, studies are conducted on a polyhydroxyalkanoate in which a cyano or nitro group is substituted on an aromatic ring in its unit, as well as on fluorine-substituted PHA in which fluorine is substituted on an aromatic ring in its unit.

[0009]

It has been reported that a polyhydroxyalkanoate containing 3-hydroxy-6-(p-cyanophenoxy)hexanoic acid or 3-hydroxy-6-(p-nitrophenoxy)hexanoic acid as a monomer unit is

produced with octanoic acid and 6-(p-cyanophenoxy)hexanoic acid or 6-(p-nitrophenoxy)hexanoic acid as substrates, using a Pseudomonas oleovorans ATCC 29347 strain and a Pseudomonas putida KT 2442 strain (see Non-Patent Literatures 7 and 8).

[0010]

Such a polyhydroxyalkanoate containing units each having an aromatic ring having a substituent group thereof can be a multifunctional polyhydroxyalkanoate, which possesses a new function derived from the substituent group existing on the aromatic ring, while maintaining polymer characteristics derived from the aromatic ring, such as a high glass transition temperature and good processability.

[0011]

At the same time, studies are vigorously conducted directed towards the obtainment of a multifunctional polyhydroxyalkanoate, which is based on a polyhydroxyalkanoate having a bromo group in its unit and obtained by introducing any given functional group into the side chain of a produced polymer by chemical transformation using the above bromo group.

[0012]

It has been reported that a polyhydroxyalkanoate having a bromo group on a side

chain thereof is produced using Pseudomonas oleovorans, and then the side chain is modified with the thiolated product of an acetylated maltose, to synthesize a polyhydroxyalkanoate having different solubility and hydrophilicity (see Non-Patent Literature 9).

[0013]

It has been reported that polyester having a vinyl group on a side chain thereof is produced using Pseudomonas oleovolans, and then the vinyl group in the polyester molecule is oxidized, so as to produce polyester having an epoxy group on its side chain (see Non-Patent Literature 10).

[0014]

It has been reported that polyester having a vinyl group on a side chain thereof is produced using Pseudomonas oleovolans, and then the vinyl group is epoxidized, to produce polyester having an epoxy group on its side chain (see Non-Patent Literature 11).

[0015]

It has been reported that using a vinyl group on the side chain of polyester, a crosslinking reaction is carried out in the polyester molecule, to improve the properties of the polyester (see Non-Patent Literature 12).

[0016]

To change the physical properties of a PHA having an active group in its unit to actually use it as a polymer, the synthesis of a PHA copolymer comprising units other than units having active groups by using microorganisms has been studied. It has been reported that using Pseudomonas oleovorans, a PHA copolymer comprising a 3-hydroxy-ω-bromoalkanoic acid unit and a straight-chain alkanoic acid unit has been produced in the coexistence of ω-bromoalkanoic acid such as 11-bromoundecanoic acid, 8-bromooctanoic acid and 6-bromohexanoic acid and n-nonanoic acid (see Non- Patent Literature 13).

[0017]

Thus, into PHA having an active group with high reactivity, such as a bromo or vinyl group, in its units, various functional groups can be introduced. Or, chemical transformation can also be performed on such PHA. Moreover, since PHA having an active group can be a crosslink point of a polymer, it can be said that such PHA is extremely effective for achievement of multifunctional PHA.

[0018]

[Patent Literature 1]

Japanese Patent No. 2989175

[Non-Patent Literature 1]

"Biodegradable Plastics Handbook" edited by Society for the Study of Biodegradable Plastics and

published from N.T.S Corp., 1995, pp.178-197

[Non-Patent Literature 2]

Macromol. Chem. Vol.191, 1990, pp.1957-1965

[Non-Patent Literature 3]

Macromolecules, Vol. 24, 1991, pp.5256-5260

[Non-Patent Literature 4]

Macromolecules, Vol. 29, 1996, pp.1762-1766

[Non-Patent Literature 5]

Macromolecules, Vol. 32, 1999, pp.2889-2895

[Non-Patent Literature 6]

Macromol. Chem. Phys., Vol. 195, 1994, pp.1665-1672

[Non-Patent Literature 7]

Can. J. Microbiol., Vol. 41, 1995, pp.32-43

[Non-Patent Literature 8]

Polymer International, Vol. 39, 1996, pp.205-213

[Non-Patent Literature 9]

Macromol. Rapid Commun., Vol. 20, 1999, pp.91-94

[Non-Patent Literature 10]

Polymer, Vol. 41, 2000, pp.1703-1709

[Non-Patent Literature 11]

Macromolecules, Vol. 31, 1998, pp.1480-1486

[Non-Patent Literature 12]

Polymer, Vol. 35, 1994, pp.2090-2097

[Non-Patent Literature 13]

Macromolecules, Vol. 25, 1992, pp.1852-1857

[0019]

[Problems to be Solved by the Invention]

However, in a case where a PHA having a bromo group as an active group is synthesized using microorganisms, the productivity of the obtained PHA is low. In a case where a PHA copolymer is synthesized using microorganisms, it is difficult to increase or control the unit ratio of bromo groups.

[0020]

Further, in the case of the synthesis of a PHA having a vinyl group as an active group, if the vinyl group is located at the end of an alkyl chain, the synthesized PHA has a low glass transition temperature and a low melting point, and therefore it cannot be said that the obtained PHA has physical properties preferable for the processability and usability of the polymer.

[0021]

For the above described reasons, a new PHA having an active group, which is produced by microorganisms with high productivity, and in which the ratio of units in its side chain having an active group can be controlled and the physical properties can be arbitrarily controlled so that its application as a polymer is not limited, and a method for producing the same, have been desired.

[0022]

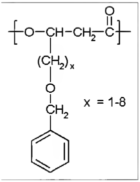
[Means for Solving the Problems]

As a result of intensive studies directed towards achieving the above object, the present inventors have found a method for synthesizing a PHA comprising a unit having a highly reactive (phenylmethyl)oxy structure as an active group by using microorganisms, thereby completing the present invention.

According to an aspect of the present invention, there is provided a polyhydroxyalkanoate comprising a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[0023]

[Chemical Formula 45]



(1)

wherein x can be one or more integers within the range shown in the chemical formula.

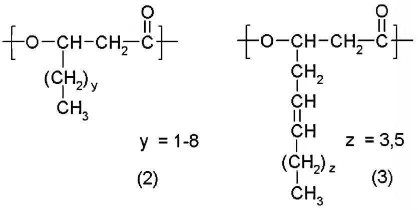
[0024]

The present invention also relates to a polyhydroxyalkanoate containing a 3-hydroxyalkanoic

acid unit expressed by chemical formula (2) or a 3-hydroxyalka-5-hydrochloric acid unit expressed by chemical formula (3) which is biosynthesized via a fatty acid synthetic system utilizing a proliferous substrate added into a medium, in addition to the unit expressed by chemical formula (1):

[0025]

[Chemical Formulae 46]

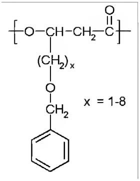


wherein y and z can be one or more integers within the range shown in the chemical formulas, while being independent from the unit expressed by chemical formula (1).

[0026]

According to the present invention, there is also provided the polyhydroxyalkanoate at least comprising simultaneously, in a molecule thereof, the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[Chemical Formula 47]

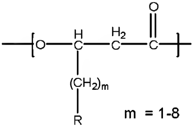


(1)

wherein x can be one or more integers within the range shown in the chemical formula, and a 3-hydroxy-alkanoic acid unit expressed by chemical formula (4):

[0027]

[Chemical Formula 48]

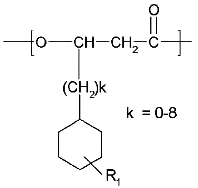


(4)

wherein m can be one or more integers within the range shown in the chemical formula, and R comprises a residue having either a phenyl structure or thienyl structure, or a 3-hydroxy-ω-cyclohexylalkanoic acid unit expressed by chemical formula (5):

[0028]

[Chemical Formula 49]



(5)

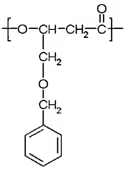
wherein R_1 is H, CN, NO_2, halogen, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and k can be one or more integers within the range shown in the chemical formula.

[0029]

The polyhydroxyalkanoate according to the present invention may be a polyhydroxyalkanoate in which the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1) is either one or both of:

a 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid unit expressed by chemical formula (6):

[Chemical Formula 50]

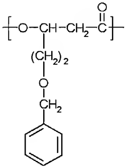


(6)

and a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit expressed by chemical formula (7):

[0030]

[Chemical Formula 51]

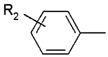


(7)

[0031]

The polyhydroxyalkanoate according to the present invention may be a polyhydroxyalkanoate in which R in chemical formula (4) is a group selected from the group consisting of

[Chemical Formula 52]

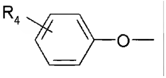


(8)

wherein R_2 is H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, $CH=CH_2$, $COOR_3$ (wherein R_3 represents any one selected from the group consisting of H, Na and K), CF_3 , C_2F_5 and C_3F_7 , and in a case where there exist a plurality of units, R_2 may be different for each unit; chemical formula (9)

[0032]

[Chemical Formula 53]

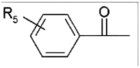


(9)

wherein R_4 is selected from the group consisting of H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, SCH_3, CF_3, C_2F_5 and C_3F_7, and in a case where there exist a plurality of units, R_4 may be different for each unit; chemical formula 10

[0033]

[Chemical Formula 54]

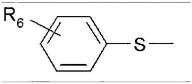


(10)

wherein R_5 is selected from the group consisting of H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and in a case where there exist a plurality of units, R_5 may be different for each unit; chemical formula 11

[0034]

[Chemical Formula 55]



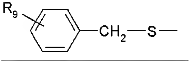
(11)

wherein R_6 is selected from the group consisting of H, halogen, CN, NO_2, $COOR_7$, SO_2R_8 (wherein R_7 represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_8 represents any one selected from the group consisting of OH, ONa, OK, halogen,

OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH, and $(CH_3)_3$-C , and in a case where there exist a plurality of units, R_6 may be different for each unit; chemical formula 12

[0035]

[Chemical Formula 56]

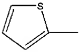


(12)

wherein R_9 represents a substituent group on the aromatic ring, R_9 is selected from thg group consisting of H, halogen, CN , NO_2 , $COOR_{10}$, SO_2R_{11} (wherein R_{10} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{11} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_9 may be different for each unit; chemical formula 13

[0036]

[Chemical Formula 57]

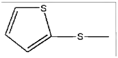


(13)

chemical formula 14

[0037]

[Chemical Formula 58]

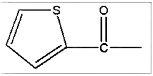


(14)

chemical formula 15

[0038]

[Chemical Formula 59]

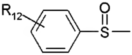


(15)

chemical formula 16

[0039]

[Chemical Formula 60]

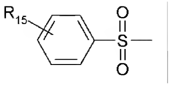


(16)

wherein R_{12} is selected from the group consisting of H, halogen, CN, NO_2, $COOR_{13}$, SO_2R_{14} (wherein R_{13} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{14} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_{12} may be different for each unit; chemical formula 17

[0040]

[Chemical Formula 61]


(17)

wherein R_{15} is selected from the group consisting of H, halogen, CN, NO_2, $COOR_{16}$, SO_2R_{17} (wherein R_{16} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{17} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_{15} may be different for each unit; and chemical formula 18

[0041]

[Chemical Formula 62]

[0042]

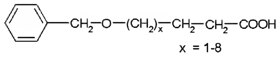
The polyhydroxyalkanoate according to the present invention may be a polyhydroxyalkanoate in which a number average molecular weight is within the range between 1,000 and 1,000,000.

[0043]

The present invention also provides a method for producing a polyhydroxyalkanoate, characterized in that the polyhydroxyalkanoate is biosynthesized

under a condition containing ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (19):

[Chemical Formula 63]

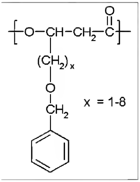


(19)

wherein x can be one or more integers within the range shown in the chemical formula, using the ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (19) as a raw material, with a microorganism with an ability to produce a polyhydroxyalkanoate comprising in a molecule thereof a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[0044]

[Chemical Formula 64]



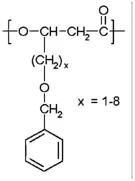
(1)

wherein x can be one or more integers within the range shown in the chemical formula, in the method of procuding the polyhydroxyalkanoate comprising in a

molecule thereof a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[0045]

[Chemical Formula 65]



(1)

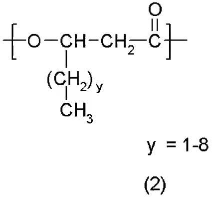
wherein x can be one or more integers within the range shown in the chemical formula.

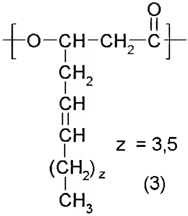
[0046]

In some cases, the polyhydroxyalkanoate may contain at least one unit expressed by the following chemical formulas (2) and (3):

[0047]

[Chemical Formulae 66]



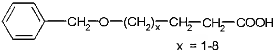


wherein y and z can be one or more integers within the range shown in the chemical formulas, while being independent from the unit expressed by chemical formula (1).

[0048]

The present invention also provides a method for producing a polyhydroxyalkanoate, characterized in that under a condition which contains a ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (19):

[Chemical Formula 67]

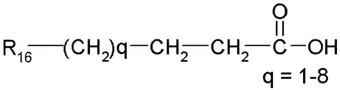


(19)

wherein x can be one or more integers within the range shown in the chemical formula, and ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (20):

[0049]

[Chemical Formula 68]

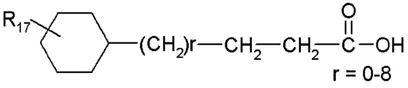


(20)

wherein q can be one or more integers within the range shown in the chemical formula, and R_{16} comprises a residue having either a phenyl structure or thienyl structure, or ω-cyclohexylalkanoic acid expressed by chemical formula (21) :

[0050]

[Chemical Formula 69]



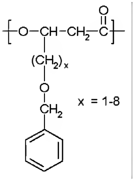
(21)

wherein R_{17} is selected from the group consisting of H, CN, NO_2, halogen, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and r can be one or more integers within the range shown in the chemical formula, using the ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (19) and the ω-cyclohexylalkanoic acid expressed by chemical formula (20) or (21) as the raw materials, the polyhydroxyalkanoate is biosynthesized with a microorganism with an ability to produce a polyhydroxyalkanoate copolymer which

contains simultaneously in a molecule thereof, the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[0051]

[Chemical Formula 70]

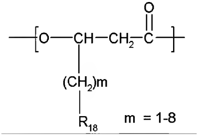


(1)

wherein x can be one or more integers within the range shown in the chemical formula, and a 3-hydroxy-alkanoic acid unit expressed by chemical formula (22):

[0052]

[Chemical Formula 71]



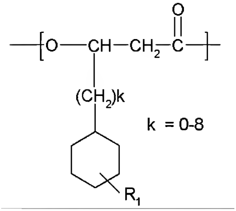
(22)

wherein m can be one or more integers within the range shown in the chemical formula, and R_{18} comprises a residue having either a phenyl structure or thienyl structure, or 3-hydroxy-ω-cyclohexylalkanoic acid

unit expressed by chemical formula (5): and

[0053]

[Chemical Formula 72]

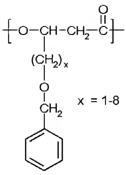


(5)

wherein R_1 is selected from the group consisting of H, CN, NO_2, halogen, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and k can be one or more integers within the range shown in the chemical formula, in the method of producing the polyhydroxyalkanoate which contains simultaneously, in its molecule, the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by chemical formula (1):

[0054]

[Chemical Formula 73]

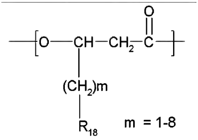


(1)

wherein x can be one or more integers within the range shown in the chemical formula, and a 3-hydroxy-alkanoic acid unit expressed by chemical formula (22):

[0055]

[Chemical Formula 74]

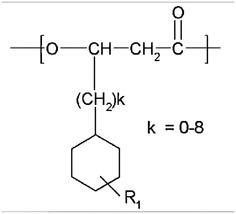


(22)

wherein m can be one or more integers within the range shown in the chemical formula, and R_{18} comprises a residue having either a phenyl structure or thienyl structure, or 3-hydroxy-ω-cyclohexylalkanoic acid unit expressed by chemical formula (5):

[0056]

[Chemical Formula 75]



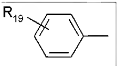
(5)

wherein R_1 is selected from the group consisting of H, CN, NO_2, halogen, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and k can be one or more integers within the range shown in the chemical formula.

The present invention also provides the method for producing a polyhydroxyalkanoate wherein R_{16} in chemical formula (20) and R_{18} in chemical formula (17), that is, residues having a phenyl structure and thienyl structure are groups expressed by chemical formula 25

[0057]

[Chemical Formula 76]



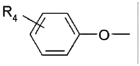
(25)

wherein R_{19} is selected from the group consisting of H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, CH=CH_2, CF_3, C_2F_5 and C_3F_7, and in a case where there exist a plurality of units, R_{19} may be different for each unit; chemical

formula 9

[0058]

[Chemical Formula 77]

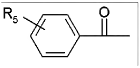


(9)

wherein R_4 is selected from the group consisting of H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, SCH_3, CF_3, C_2F_5 and C_3F_7, and in a case where there exist a plurality of units, R_4 may be different for each unit; chemical formula 10

[0059]

[Chemical Formula 78]

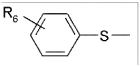


(10)

wherein R_5 is selected from the group consisting of H, halogen, CN, NO_2, CH_3, C_2H_5, C_3H_7, CF_3, C_2F_5 and C_3F_7, and in a case where there exist a plurality of units, R_5 may be different for each unit; chemical formula 11

[0060]

[Chemical Formula 79]



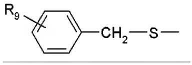
(11)

wherein R_6 is selected from the group consisting of H, halogen, CN, NO_2, $COOR_7$, SO_2R_8 (wherein R_7 represents

any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_8 represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_6 may be different for each unit; chemical formula 12

[0061]

[Chemical Formula 80]

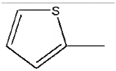


(12)

wherein R_9 is selected from the group consisting of H, halogen, CN, NO_2, $COOR_{10}$, SO_2R_{11} (wherein R_{10} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{11} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_9 may be different for each unit; chemical formula 13

[0062]

[Chemical Formula 81]

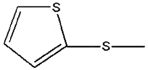


(13)

chemical formula 14

[0063]

[Chemical Formula 82]

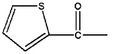


(14)

chemical formula 15

[0064]

[Chemical Formula 83]

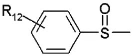


(15)

chemical formula 16

[0065]

[Chemical Formula 84]

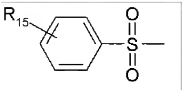


(16)

wherein R_{12} is selected from the group consisting of H, halogen, CN, NO_2, $COOR_{13}$, SO_2R_{14} (wherein R_{13} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{14} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3, C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_{12} may be different for each unit; chemical formula 17

[0066]

[Chemical Formula 85]



(17)

wherein R_{15} is selected from the group consisting of H, halogen, CN, NO_2, $COOR_{16}$, SO_2R_{17} (wherein R_{16} represents any one selected from the group consisting of H, Na, K, CH_3 and C_2H_5, and R_{17} represents any one selected from the group consisting of OH, ONa, OK, halogen, OCH_3 and OC_2H_5), CH_3 , C_2H_5, C_3H_7, $(CH_3)_2$-CH and $(CH_3)_3$-C, and in a case where there exist a plurality of units, R_{15} may be different for each unit; and chemical formula 18

[0067]

[Chemical Formula 86]

.

[0068]

The present invention also provides the method for producing a polyhydroxyalkanoate wherein the microorganisms is cultured in a medium containing ω-[(phenylmethyl)oxy]alkanoic acid expressed by chemical formula (19).

[0069]

The present invention also provides the method for producing a polyhydroxyalkanoate wherein the medium contains at least one selected from the group consisting of peptides, yeast extract, organic acids or salts thereof, amino acids or salts thereof, saccharides and straight-chain alkanoic acids , which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms or salts thereof. The present invention further provides the method for producing a polyhydroxyalkanoate wherein the peptide is polypeptone; the organic acids or salts thereof are one or more compounds selected from the group consisting of pyruvic acid, oxaloacetic acid, citric acid, isocitric acid, ketoglutaric acid, succinic acid, fumaric acid, malic acid, lactic acid, and salts thereof; the amino acids or salts thereof are one or more compounds selected from the group consisting of glutamic acid, aspartic acid, and salts thereof; and the saccharides are one or more compounds selected from the group consisting of glyceroaldehyde, erythrose, arabinose, xylose, glucose, galactose, mannose, fructose, glycerol, erythritol, xylitol, gluconic acid, glucuronic acid and galacturonic acid, maltose, sucrose and lactose.

[0070]

Detailed culture conditions of microorganisms in the method for producing the polyhydroxyalkanoate

of the present invention are as follows:

[0071]

As described below, various necessary substrates and nutrients are added to an inorganic salt medium basically containing a phosphate buffer and ammonium salts or nitrates.

[0072]

In order to produce a polyhydroxyalkanoate of interest expressed by the above chemical formula (1) comprising a 3-hydroxy-ω-(phenylmethyl)alkanoic acid unit, it is desirable that ω-[(phenylmethyl)oxy]alkanoic acid expressed by the above chemical formula (19) is contained in the medium as a substrate, at a proportion from 0.01% to 1% (w/v) per medium, and more preferably at a proportion from 0.02% to 0.2% per medium.

[0073]

In order to produce a polyhydroxyalkanoate comprising in a molecule thereof a 3-hydroxy-alkanoic acid unit expressed by chemical formula (22) or a 3-hydroxy-ω-cyclohexylalkanoic acid unit expressed by chemical formula (5) as well as a 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit, it is desirable that each of ω-[(phenylmethyl)oxy]alkanoic acid of the above chemical formula (19) and an alkanoic acid expressed by chemical formula (20) or ω-cyclohexylalkanoic acid expressed by the above

chemical formula (21) is contained as a substrate in the medium at a proportion from 0.01% to 1% (w/v) per medium, and more preferably at a proportion from 0.02% to 0.2% per medium.

[0074]

A carbon source and a nitrogen source for growth of microorganisms and for production of a polyhydroxyalkanoate are preferably added to the medium at a concentration from 0.1% to 5% (w/v) per medium, and more preferably from 0.2% to 2% per medium.

[0075]

Any medium can be used in the present invention, as long as it is an organic salt medium containing phosphate and a nitrogen source such as ammonium salts or nitrates. It is possible to increase the productivity of PHA by controlling the concentration of the nitrogen source.

[0076]

Any temperature is applied as a culture temperature, as long as the above cell strains can grow favorably at the temperature. A temperature between 15°C and 37°C is appropriate, and a temperature between 20°C and 30°C is more preferable.

[0077]

Any culturing method such as a liquid culturing method, a solid culturing method or the like can be

used, as long as microorganisms can grow and produce PHA by this method. Any type of culture, such as a batch culture, a fed-batch culture, a semi-continuous culture, a continuous culture or the like can be used with no limitation. A liquid batch culture can be embodied into a method of shaking a medium in a flask to supply oxygen thereto and an oxygen supplying method of a stirring aeration system using a jar fermenter.

[0078]

In addition to the above described methods, there is another method for allowing microorganisms to produce and accumulate PHA. This method comprises making microorganism sufficiently grow, transferring the cells to a medium in which a nitrogen source such as ammonium chloride is limited, and further culturing them in a state where a compound as a substrate of a unit of interest is present. This method improves productivity in some cases.

[0079]

Moreover, after the microorganisms are cultured under the above described conditions, the method may comprise a step of recovering a polyhydroxyalkanoate comprising the 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by the above chemical formula (1) produced by the above microorganisms from the cells.

[0080]

As a method of recovering a PHA of interest from the cells of microorganisms, a common method can be adopted. For example, extraction with an organic solvent such as chloroform, dichloromethane, ethylacetate or acetone is the most simple, but dioxane, tetrahydrofuran or acetonitrile may also be used in some cases. In an environment in which the use of organic solvents is not preferred, any one of a treatment with surfactants such as SDS, a treatment with an enzyme such as lysozyme, a treatment with chemicals such as hypochlorite, ammonium or EDTA, an ultrasonic crushing method, a homogenizer method, a pressure crushing method, a bead impulse method, a grinding method, an immersion method and a freeze-thaw method may be used to physically crush microorganism cells. Cell components other than PHA are removed by any one of the above methods, to collect PHA.

[0081]

As microorganisms used for the production method of the present invention, any species of microorganisms may be used, as long as they have an ability to satisfy the above described conditions. Among them, microorganisms belonging to Pseudomonas species are desirable. Specific examples of preferred species include Pseudomonas cichorii,

Pseudomonas putida, Pseudomonas fluorecense, Pseudomonas oleovorans, Pseudomonas aeruginosa, Pseudomonas stutzeri, and Pseudomonas jessenii. More specifically, examples of a suitable strain include Pseudomonas cichorii YN2 (FERM BP-7375), Pseudomonas cichorii H45 (FERM BP-7374), Pseudomonas jessenii P161 (FERM BP-7376)n and Pseudomonas putida P91 (FERM BP-7373. These four types of strains were deposited at the International Patent Organism Depositary (IPOD) of Life Engineering Technological Institute of National Institute of Advanced Industrial Science and Technology (AIST) (the former Ministry of International Trade and Industry, Industrial Technological Laboratory) and they are described in Japanese Patent Application No. H11-371863.

[0082]

It should be noted that the culture of microorganisms in the present invention, the production of PHA by microorganisms and accumulation in the cells in the present invention, and the recovery of PHA from the cells in the present invention are not limited to the above described methods.

[0083]

[Embodying Aspects of the Invention]

The composition of an inorganic salt culture medium (M9 medium) used in one method of the present

invention is shown below.

[0084]

[M9 medium]

Na_2HPO_4: 6.3

KH_2PO_4: 3.0

NH_4Cl: 1.0

NaCl: 0.5 g/L, pH = 7.0

[0085]

To ensure satisfactory growth of cells and associated good productivity of PHA, it is necessary to add an approximately 0.3% (v/v) trace component solution shown below to the above inorganic salt medium.

[0086]

[Trace component solution]

nitrilotriacetic acid: 1.5; $MgSO_4$: 3.0; $MnSO_4$: 0.5; NaCl: 1.0; $FeSO_4$: 0.1;

$CaCl_2$: 0.1; $CoCl_2$: 0.1; $ZnSO_4$: 0.1; $CuSO_4$: 0.1; $AlK(SO_4)_2$: 0.1; H_3BO_3: 0.1; Na_2MoO_4: 0.1; $NiCl_2$: 0.1 g/L

[0087]

Examples

[Example 1]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking

the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0088]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 33 mg of PHA.

[0089]

An NMR analysis was carried out on the obtained PHA under the following conditions:

<Measuring equipment> FT-NMR: Bruker DPX400

Resonance frequency: ^{1}H = 400 MHz

<Measuring equipment> Type of nuclear species: ^{1}H

Used solvent: $CDCl_3$

Measuring temperature: room temperature

Figure 1 shows a ^{1}H-NMR spectrum chart, and Table 1 shows the identification results.

[0090]

Table 1

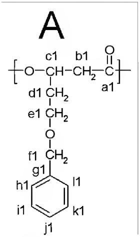
Chemical shift (ppm)	Attribution	Fragmentation	Integration ratio
1.86	d1	m	2H
2.54	b1	m	2H
3.44	e1	m	2H
4.41	f1	s	2H
5.31	c1	m	1H
7.20 to 7.31	h1 i1 j1 k1 l1	m	5H

[0091]

As shown in Table 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 94.9 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0092]

[Chemical Formula 87]



(26)

[0093]

Furthermore, the molecular weight of the obtained PHA was evaluated by gel permeation chromatography (GPC: Tosoh HLC-8220, column: Tosoh TSK-GEL Super HM-H, solvent: chloroform, polystyrene conversion). As a result, Mn = 123,000, and Mw = 293,000.

[0094]

[Example 2]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium, which contained 0.5% D-glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid but did not contain a nitrogen source (NH_4Cl), followed by further shaking the resulting liquid at

30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0095]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 30 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 92.6 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0096]

[Example 3]

A Pseudomonas cichorii H45 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone, and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0097]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 31 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula

(26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 91.6 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0098]

[Example 4]

A Pseudomonas jessenii P161 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone, and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0099]

The obtained lyophilized pellet was suspended

in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 29 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 90.8 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0100]

[Example 5]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone, and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking

the medium at 30°C at 125 strokes/minute. At 48 hours later, 20 mL of aqueous solution containing 5% D-glucose and 1% 5-[(phenylmethyl)oxy]valeric acid was added thereto, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0101]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 18 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-

hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 79.7 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0102]

[Example 6]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% polypeptone and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium, which contained 0.5% pyruvic acid and 0.1% 5-[(phenylmethyl)oxy]valeric acid but did not contain a nitrogen source (NH_4Cl), followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0103]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained

condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 16 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 77.8 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0104]

[Example 7]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% polypeptone and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, fungus cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0105]

The obtained lyophilized pellet was suspended

in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 35 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 74.2 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0106]

[Example 8]

A Pseudomonas cichorii H45 strain was inoculated into 200 mL of M9 medium containing 0.5% yeast extract and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125

strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0107]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 30 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 75.9 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0108]

[Example 9]

A Pseudomonas jessenii P161 strain was inoculated into 200 mL of M9 medium containing 0.5% glucose and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0109]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 28 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-

hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 81.5 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0110]

[Example 10]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% pyruvic acid and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0111]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 15 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-

[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 88.5 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0112]

[Example 11]

A Pseudomonas cichorii H45 strain was inoculated into 200 mL of M9 medium containing 0.5% sodium glutamate and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0113]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected

to vacuum drying, to obtain 24 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 86.3 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0114]

[Example 12]

A Pseudomonas jessenii P161 strain was inoculated into 200 mL of M9 medium containing 0.1% nonanoic acid and 0.1% 5-[(phenylmethyl)oxy]valeric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0115]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The

extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 11 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA was a PHA expressed by chemical formula (26), which comprised 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 24.5 mol% 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid as a monomer unit.

[0116]

[Example 13]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone, and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and

they were resuspended in 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0117]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 30 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the

obtained PHA comprised 92.4 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0118]

Furthermore, the molecular weight of the obtained PHA was evaluated by gel permeation chromatography (GPC: Tosoh HLC-8220, column: Tosoh TSK-GEL Super HM-H, solvent: chloroform, polystyrene conversion). As a result, Mn = 138,000, and Mw = 294,000.

[0119]

[Example 14]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium, which contained 0.5% D-glucose and 0.1% 4-[(phenylmethyl)oxy]butyric acid but did not contain a nitrogen source (NH_4Cl), followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0120]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was

stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 26 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 90.5 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[9121]

[Example 15]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% D-glucose, 0.1% polypeptone, and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, 20 mL of aqueous solution containing 5% D-

glucose and 1% 4-[(phenylmethyl)oxy]butyric acid was added thereto, followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0122]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 14 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 76.8 mol% 3-hydroxy-4-

[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0123]

[Example 16]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% polypeptone and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation, and they were resuspended in 200 mL of M9 medium, which contained 0.5% pyruvic acid and 0.1% 4-[(phenylmethyl)oxy]butyric acid but did not contain a nitrogen source (NH_4Cl), followed by further shaking the resulting liquid at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0124]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 14 mg of PHA. As a

result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 73.2 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0125]

[Example 17]

A Pseudomonas cichorii YN2 strain was inoculated into 200 mL of M9 medium containing 0.5% polypeptone and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0126]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was

concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 31 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 76.7 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0127]

[Example 18]

A Pseudomonas cichorii H45 strain was inoculated into 200 mL of M9 medium containing 0.5% yeast extract and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0128]

The obtained lyophilized pellet was suspended

in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 29 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 75.5 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0129]

[Example 19]

A Pseudomonas jessenii P161 strain was inoculated into 200 mL of M9 medium containing 0.5% glucose and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were

recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0130]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 26 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 85.9 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0131]

[Example 20]

A Pseudomonas cichorii YN2 strain was

inoculated into 200 mL of M9 medium containing 0.5% pyruvic acid and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0132]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 11 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 90.4 mol% 3-hydroxy-4-

[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0133]

[Example 21]

A Pseudomonas cichorii H45 strain was inoculated into 200 mL of M9 medium containing 0.5% sodium glutamate and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0134]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 27 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12

carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 84.3 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0135]

[Example 22]

A Pseudomonas jessenii P161 strain was inoculated into 200 mL of M9 medium containing 0.1% nonanoic acid and 0.1% 4-[(phenylmethyl)oxy]butyric acid, followed by shaking the medium at 30°C at 125 strokes/minute. At 48 hours later, cells were recovered by centrifugation and washed once with cold methanol, and then lyophilized.

[0136]

The obtained lyophilized pellet was suspended in 20 mL of chloroform, and the suspension was stirred at 60°C for 20 hours to extract PHA. The extract was filtered through a membrane filter with a pore size of 0.45 μm, and the filtrate was concentrated using a rotary evaporator. The obtained condensate was reprecipitated in cold methanol, and then only the precipitate was recovered and subjected to vacuum drying, to obtain 8 mg of PHA. As a result of carrying out an NMR analysis under the same conditions as in Example 1, it was found that the obtained PHA comprised 3-hydroxy-4-

[(phenylmethyl)oxy]butyric acid as a monomer unit and further comprised, as a monomer unit, 3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid. Moreover, from the ^{1}H-NMR spectrum integration ratio, it was found that the obtained PHA comprised 21.9 mol% 3-hydroxy-4-[(phenylmethyl)oxy]butyric acid as a monomer unit.

[0137]

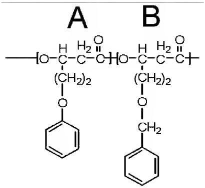
[Example 23]

0.5% glucose, 6 mM 5-phenoxyvaleric acid, and 3 mM 5-[(phenylmethyl)oxy]valeric acid were dissolved in 100 ml of the above M9 medium, and the resultant solution was placed in a 200 ml shaking flask and was then sterilized with an autoclave, followed by cooling to room temperature. 2 ml of the culture solution of a Pseudomonas cichorii YN2 strain that had previously been subjected to shaking culture at 30°C for 8 hours in an M9 medium containing 0.5% polypeptone was added to the above prepared medium, followed by culture at 30°C for 48 hours. After completion of the culture, cells were recovered, and the thus obtained cells were resuspended in 100 ml of the same above medium, followed by culture in a 200 ml shaking flask at 30°C for 42 hours. After completion of the culture, cells were recovered by

centrifugation and washed with methanol, and then dried. After weighing the dried cells, chloroform was added thereto, and the mixture was stirred at 35°C for 72 hours, to extract a polymer. The chloroform containing the extracted polymer was filtered, and the filtrate was concentrated using an evaporator. Thereafter, the precipitated and solidified portion was collected in cold methanol, and the portion was dried under reduced pressure, to obtain a polymer of interest. Figure 2 shows the results obtained from an NMR analysis that was carried out under the same conditions as in Example 1. It was confirmed that the obtained PHA was a polyhydroxyalkanoate copolymer comprising the units expressed by the following chemical formula (27) (A : B : other units (3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid) = 63 : 37 : 0). Moreover, it was confirmed by ^{13}C-NMR (<measuring equipment> FT-NMR: Bruker DP x 400, resonance frequency: ^{13}C = 100 MHz, <measuring equipment> type of nuclear species: ^{13}C, used solvent: $CDCl_3$, measuring temperature: room temperature) that the obtained PHA comprised unit B, that is, a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit.

[0138]

[Chemical Formula 88]



(27)

[0139]

The molecular weight of the polymer was determined by gel permeation chromatography (GPC) (GPC: Tosoh HLC-8220, column: Tosoh TSK-GEL Super HM-H, solvent: chloroform, polystyrene conversion).

[0140]

The weight of the obtained polymer (PDW) was 0.17 g/l, and the number average molecular weight of the obtained polymer was 93,000.

[0141]

[Example 24]

0.5% glucose, 0.1% polypeptone, 6 mM 5-phenoxyvaleric acid, and 3 mM 5-[(phenylmethyl)oxy]valeric acid were dissolved in 100 ml of the above M9 medium, and the resultant solution was placed in a 200 ml shaking flask and was then sterilized with an autoclave, followed by cooling to room temperature. 2 ml of the culture solution of a Pseudomonas cichorii YN2 strain that had previously

been subjected to shaking culture at 30°C for 8 hours in an M9 medium containing 0.5% polypeptone was added to the above prepared medium, followed by culture at 30°C for 42 hours. After completion of the culture, cells were recovered and washed with methanol, and then dried. After weighing the dried cells, chloroform was added thereto, and the mixture was stirred at 35°C for 72 hours, to extract a polymer. The chloroform containing the extracted polymer was filtered, and the filtrate was concentrated using an evaporator. Thereafter, the precipitated and solidified portion was collected in cold methanol, and the portion was dried under reduced pressure, to obtain a polymer of interest.

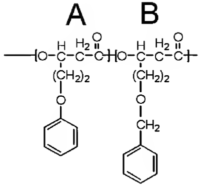
[0142]

To determine the structure of the obtained polymer, ^{1}H-NMR was carried out in the same manner as in Example 1. As a result, it was confirmed that the obtained polymer was a polyhydroxyalkanoate copolymer comprising the units expressed by the following chemical formula (27) (A : B : other units (3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid) = 38 : 33 : 29). Moreover, by ^{13}C-NMR, it was confirmed that the obtained polymer comprised unit B, that is, a 3-hydroxy-5-

[(phenylmethyl)oxy]valeric acid unit.

[0143]

[Chemical Formula 89]



(27)

[0144]

The molecular weight of the polymer was determined by GPC in the same manner as in Example 1.

[0145]

The weight of the obtained polymer (PDW) was 0.06 g/l, and the number average molecular weight of the obtained polymer was 94,000.

[0146]

[Example 25]

A polymer of interest was obtained by the same method as in Example 24 with the exceptions that a Pseudomonas cichorii H45 strain was used instead of the YN2 strain used in Example 24, and that 0.5 % yeast extract was used instead of glucose and polypeptone used in Example 24.

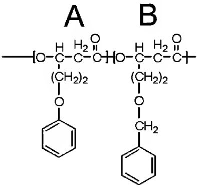
[0147]

To determine the structure of the obtained

polymer, ^{1}H-NMR was carried out in the same manner as in Example 1. As a result, it was confirmed that the obtained polymer was a polyhydroxyalkanoate copolymer comprising the units expressed by the following chemical formula (27) (A : B : other units (3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid) = 42 : 33 : 25). Moreover, ^{13}C-NMR was carried out and as a result, it was confirmed that the obtained polymer comprised unit B, that is, a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit.

[0148]

[Chemical Formula 90]



(27)

[0149]

As with Example 1, the molecular weight of the obtained polymer was determined by GPC.

[0150]

The weight of the obtained polymer (PDW) was 0.05 g/l, and the number average molecular weight of

the obtained polymer was 91,000.

[0151]

[Example 26]

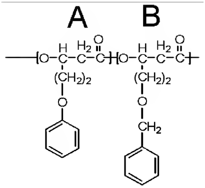
A polymer of interest was obtained by the same method as in Example 24 with the exceptions that a Pseudomonas cichorii H45 strain was used instead of the YN2 strain used in Example 24, and that 0.5 % sodium pyruvate was used instead of glucose and polypeptone used in Example 24.

[0152]

To determine the structure of the obtained polymer, ^{1}H-NMR was carried out in the same manner as in Example 1. As a result, it was confirmed that the obtained polymer was a polyhydroxyalkanoate copolymer comprising the units expressed by the following chemical formula (27) (A : B : other units (3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid) = 58 : 24 : 18). Moreover, ^{13}C-NMR was carried out, and as a result, it was confirmed that the obtained polymer comprised unit B, that is, a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit.

[0153]

[Chemical Formula 91]



(27)

[0154]

As with Example 1, the molecular weight of the obtained polymer was determined by GPC.

[0155]

The weight of the obtained polymer (PDW) was 0.03 g/l, and the number average molecular weight of the obtained polymer was 102,000.

[0156]

[Example 27]

A polymer of interest was obtained by the same method as in Example 24 with the exceptions that a Pseudomonas jessenii P161 strain was used instead of the YN2 strain used in Example 24, and that 0.5 % sodium glutamate was used instead of glucose and polypeptone used in Example 24.

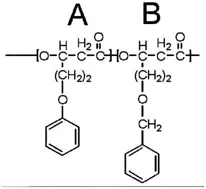
[0157]

To determine the structure of the obtained polymer, ^{1}H-NMR was carried out in the same manner as in Example 1. As a result, it was confirmed that the obtained polymer was a polyhydroxyalkanoate copolymer

comprising the units expressed by the following chemical formula (27) (A : B : other units (3-hydroxyalkanoic acid or 3-hydroxyalkenoic acid, which is saturated or unsaturated fatty acid having 4 to 12 carbon atoms, such as 3-hydroxybutyric acid or 3-hydroxyvaleric acid) = 40 : 35 : 25). Moreover, ^{13}C-NMR was carried out, and as a result, it was confirmed that the obtained polymer comprised unit B, that is, a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit.

[0158]

[Chemical Formula 92]



(27)

[0159]

As with Example 1, the molecular weight of the obtained polymer was determined by GPC.

[0160]

The weight of the obtained polymer (PDW) was 0.08 g/l, and the number average molecular weight of the obtained polymer was 89,000.

[0161]

[Example 28]

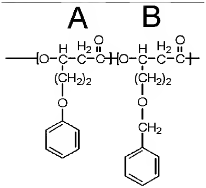
A polymer of interest was obtained by the same method as in Example 24 with the exceptions that a Pseudomonas jessenii P161 strain was used instead of the YN2 strain used in Example 24, and that 0.1% nonanoic acid was used instead of 0.5% polypeptone used in Example 24.

[0162]

To determine the structure of the obtained polymer, ^{1}H-NMR was carried out in the same manner as in Example 1. As a result, it was confirmed that the obtained polymer was a polyhydroxyalkanoate copolymer comprising the units expressed by the following chemical formula (27) (A : B : other units = 40 : 35 : 25). Moreover, ^{13}C-NMR was carried out and as a result, it was confirmed that the obtained polymer comprised unit B, that is, a 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid unit.

[0163]

[Chemical Formula 93]



(27)

[0164]

As with Example 1, the molecular weight of the obtained polymer was determined by GPC.

[0165]

The weight of the obtained polymer (PDW) was 0.04 g/l, and the number average molecular weight of the obtained polymer was 98,000.

[0166]

[Effect of the Invention]

According to the present invention, there can be provided a polyhydroxyalkanoate which is a novel polyhydroxyalkanoate copolymer, containing a unit having a [(phenylmethyl)oxy] structure in a side chain thereof, and a polyhydroxyalkanoate containing simultaneously, in a molecule, a unit having a [(phenylmethyl)oxy] structure in a side chain thereof and a unit containing a residue having any one of a phenyl structure, a thienyl structure and a cyclohexyl structure.

[0167]

In addition, there can be provided a PHA producing method by which high productivity of PHA can be attained and the ratio of units in its side chain having the [(phenylmethyl)oxy] structure can be controlled and the physical properties of the produced PHA can be controlled.

[Brief Description of the Drawings]

[Figure 1]

A ^{1}H-NMR spectrum chart of a polyhydroxyalkanoate in Example 1.

[Figure 2]

A ^{1}H-NMR spectrum chart of a polyester obtained in Example 23.

[Name of the Document] Abstract

[Abstract]

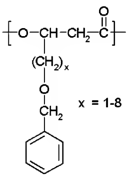
[Subject]

Provided are a PHA having an active group, which is produced by microorganisms with high productivity, and in which the ratio of units on its side chain having an active group can be controlled and the physical properties can be arbitrarily controlled so that its application as a polymer is not limited, and a method for producing the same.

[Solving Means]

A 3-hydroxy-ω-[(phenylmethyl)oxy]alkanoic acid unit expressed by the following chemical formula (1):

[Chemical Formula 1]



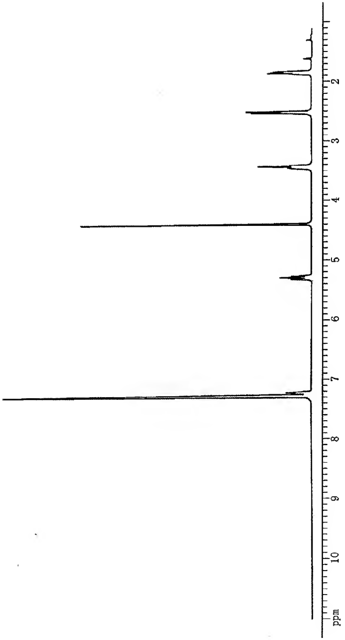
(1)

wherein x can be one or more integers within the range shown in the chemical formula.

[Elected Drawing] None

【書類名】　図面　[Name of the Document] Drawings

【図１】　[Fig. 1]



【図２】　[Fig. 2]